MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures

SOP #: MDNR-WQMS-207 EFFECTIVE DATE: August 8, 2003

SOP TITLE: Laboratory Extraction Procedure for Chlorophyll Analysis by Fluorometric Techniques

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SUMMARY OF REVISIONS: Revisions were made to convert from acetone extraction to ethanol extraction.

APPLICABILITY: The procedures outlined in this SOP apply to all ESP personnel who perform chlorophyll analysis using the Turner Designs TD-700 fluorometer.

DISTRIBUTION: MDNR Intranet
ESP SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

Laboratory extraction of chlorophyll a is the intermediate procedure between field collection and filtration of phytoplankton or periphyton samples (MDNR-WQMS-015) and fluorometric analysis of chlorophyll a (MDNR-WQMS-110). These procedures remove chlorophyll a from intact freeze-dried algae cells and places it in a solution that can be analyzed fluorometrically.

2.0 SUMMARY OF PROCEDURES

ESP uses methods for this procedure similar to those used at the University of Missouri (MU) and other laboratories in Missouri. Known amounts of buffered ethanol are used to extract chlorophyll a from freeze-dried alga cells collected on glass fiber filters.

3.0 SPECIAL CONSIDERATIONS

3.1 Because chlorophyll is easily broken down in the presence of light, conduct all handling and extraction of samples in subdued light.

3.2 Because skin secretions break down chlorophyll, wear nitrile or rubber gloves while handling samples and any materials and supplies coming into contact with samples.

3.3 When handling glass fiber filters, be careful not to disturb the surface in a way that could cause loss of sample. Using forceps to handle the outer unexposed portion of the filter is recommended.

3.4 Thoroughly triple-rinse all reusable materials coming into contact with chlorophyll a samples with deionized water and then triple rinse with 95% buffered ethanol.

4.0 HEALTH AND SAFETY REQUIREMENTS

Wear personal protective equipment (PPE) including protective gloves and safety glasses. Because the ethanol contains toxic denaturing agents and is flammable, refer to the Material Safety Data Sheet for health and safety requirements when handling denatured ethanol.

5.0 SUPPLIES AND EQUIPMENT

- buffered water (saturated MgCO3 solution in deionized water)
- 95% buffered ethanol (95% research grade ethanol, 5% buffered water)
- rubber or nitrile gloves
- forceps
- logbook

- pencil
- permanent felt tip marker
- bottles (40-50 ml) or vials (15-20 ml) with caps
- pipettes
- incubator

6.0 PROCEDURES

6.1 Phytoplankton

6.1.1 Set up logbook to record sample numbers, volume of water sample filtered in the field, volume of 95% buffered ethanol used for extraction, and any other important information.

6.1.2 Carefully peel open the glass fiber filter to allow better exposure of the side containing phytoplankton to the ethanol. Roll the glass fiber, place it into a 15 to 20 ml vial, and add 10 ml of 95% buffered ethanol with pipette. Make sure the filter is completely submerged in ethanol solution. Cap tightly and label the vial with the sample number using a permanent felt tip marker.

6.1.3 Incubate the vial(s) for fifteen minutes at 60º C. After the incubation period, place the vial(s) in the dark at room temperature for at least two hours.

6.1.4 Conduct analysis as soon as possible after performing step 6.1.3.

6.2 Periphyton

6.2.1 Set up logbook to record sample numbers, area of sample filtered in the field, volume of 95% buffered ethanol used for extraction, and any other important information

6.2.2 Carefully peel open the glass fiber filter to allow better exposure of the side containing periphyton to the ethanol. Place the glass filter in a 40 to 50 ml bottle. Using a pipette, add at least 20 ml of 95% buffered ethanol. Record volume of buffered ethanol added in logbook and make sure filter is completely submerged in ethanol. Cap tightly and label with the sample number.

6.2.3 Place vial(s) in refrigerator and keep dark for at least 48 hours before analysis. Just prior to analysis, allow vial(s) to reach room temperature. Conduct analysis as soon as possible after 48-hour extraction period according to MDNR-WQMS-110.

6.2.4 If necessary in some circumstances, especially when periphyton is collected from natural substrate in the form of heavy filamentous growth, grind the sample as recommended in section 10200 H of Standard Methods for the Examination of Water and Wastewater, 20th Edition. Conduct analysis as soon as possible after grinding and extraction according to MDNR-WQMS-110.

7.0 REFERENCES

MDNR Environmental Services Program, MDNR-WQMS-110, *Instrument Calibration and Fluorometric Determination of Chlorophyll a Using the Turner Designs Fluorometer (Model TD-700)*

MDNR Environmental Services Program, MDNR-WQMS-015, *Sample Collection and Field Handling Procedures for Chlorophyll Analysis of Surface Water Samples*

Standard Methods for the Examination of Water and Wastewater, 20th Edition Section 10200 H